

DEVELOPMENT OF A SELECTION TO RECOVER IMPROVED DNA LIGASE ENZYMES DURING DIRECTED EVOLUTION

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DNA ligases are essential enzymes used in many molecular biology applications. Of particular note, they are important enzymes in next generation sequencing (NGS) technologies. The improved speed, efficiency, and affordability of NGS over Sanger sequencing has greatly expanded the applications of DNA sequencing. In most NGS technologies ligase enzymes play a crucial role, for instance in ligating adaptors onto sequence fragments during sample preparation. This key step requires a blunt-ended ligation reaction, with highly efficient ligases required in order to create a sample library of high quality. The current go-to enzyme is T4 DNA ligase, which has not evolved in Nature to perform blunt ended ligations, and as such has relatively poor levels of activity when compared to other substrates. There is therefore potential to improve upon this enzyme and engineer a ligase that is more efficient with blunt-ended substrates. We have developed a novel function-based directed evolution selection to evolve blunt-ended ligases that have greater catalytic efficiency. The basis for this approach is the over-expression of a ligase enzyme variant which is then incubated with a linearised plasmid encoding for that same ligase variant. More efficient ligases will ligate the plasmid encoding for their own gene variant more efficiently (in a blunt-ended ligation), and so greater numbers of the circularised plasmid will be produced. Through successive rounds of transformation, amplification and ligation the most improved enzyme variants are enriched. This selection approach is being used to evaluate a panel of ligase variants in order to identify the best ligases for blunt-ended ligation applications.

